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I, JANENE PEISKER, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002952602 for a patent by VARIAN AUSTRALIA PTY LTD as filed on 12 November 2002.

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PROVISIONAL SPECIFICATION

APPLICANT: **VARIAN AUSTRALIA PTY LTD**

Invention Title: ***"FLOW THROUGH CELL FOR OPTICAL SPECTROSCOPY"***

The invention is described in the following statement:

FLOW THROUGH CELL FOR OPTICAL SPECTROSCOPY

Technical Field

The present invention relates to a flow through cell for use in a spectrophotometer for analysis of dissolved substances in a flowing liquid stream. The invention is particularly useful with high performance liquid chromatography.

Background

The following discussion of the background to the invention is included to explain the context of the invention. This is not to be taken as an admission that any of the material referred to was published, known or part of the common general knowledge in Australia as at the priority date established by the present application.

It is frequently useful to detect, and to determine the concentrations of, various dissolved chemical substances present in varying concentrations in a flowing liquid stream such as, for example, the effluent of a high-performance liquid chromatograph. If such chemical substances absorb light of characteristic wavelengths they can be detected by spectrophotometry. It is convenient to pass the flowing liquid stream (or a representative portion thereof) through a flow through cell for continuous spectrophotometric measurements. To obtain good resolution of different chemical substances eluted from the column of a chromatograph it is important that the effluent stream should undergo as little mixing as possible in its passage through the flow through cell. This is favoured by keeping the volume of the cell small and by ensuring that all parts of the conduit or passage through the cell are efficiently swept by the flowing liquid. An example of a flow cell having such characteristics is disclosed by Berick and Magnussen, Jr. in US Patent 4,374,620 (February 22, 1983). Another example is disclosed by Magnussen, Jr. in US Patent 5,064,287 (November 12, 1991). In the flow cell of each of these disclosures, means are provided adjacent the entrance end of a flow through passage and surrounding that passage to modify the flow characteristics of a liquid entering the passage. Such means evenly distributes the flow circumferentially as it enters the passage to thereby reduce

undesirable mixing of the flow. However both of these flow through cells are difficult to manufacture.

Disclosure of the Invention

An object of the present invention is to provide a relatively easily manufacturable flow through cell for spectrophotometry in which each successive increment of liquid entering the cell efficiently sweeps or flushes out the previous increment of liquid with minimal mixing.

A further object is to provide an embodiment of the invention having the characteristics just described and also having in the liquid flow path two optically separate pathways of different lengths, so that if the concentration of a chemical substance is such that it produces an absorbance that is either too high or too low to be measured accurately in one optical pathway a more appropriate absorbance will be measurable in another.

According to the invention there is provided a flow through cell for use in a spectrophotometer for analysis of dissolved chemical substances in a flowing liquid stream, including

a plurality of body members clamped together and providing a small volume flow through passage,

the body members also providing an optical pathway through a part of the flow through passage which is located between optically transparent windows associated with spaced apart body members, said part of the flow through passage comprising a hole passing through an intermediate body member together with a liquid inlet region at one end of the hole and a liquid outlet region at the other end of the hole,

wherein the liquid inlet and liquid outlet regions are provided by respectively, a portion of the flow through passage through which liquid flows into or out of a said region substantially immediately adjacent the optically transparent window transversely of the direction of the hole.

In one embodiment of the invention each respective said portion of the flow through passage is provided by a gallery in a resilient gasket located

between facing surfaces of the intermediate body member and one of the spaced apart body members wherein the gallery provides a liquid flow path between the hole and an offset inlet/outlet duct in a body member. The gaskets provide a seal between the body members when they are clamped together.

In another embodiment of the invention each respective said portion of the flow through passage is provided by a gallery formed in a body member wherein the gallery provides a liquid flow path between the hole and an offset inlet/outlet duct in a body member. In this embodiment, resilient gaskets may also be provided to seal between adjacent body members when they are clamped together, however such gaskets may be omitted by providing for at least one of two adjacent body members to be formed from a resilient material which will provide the requisite seal when these body members are clamped together. In an embodiment without gaskets, preferably the intermediate body member is made of a suitably resilient material.

In this specification, the word "gallery" is to be understood as defining a space in either a resilient gasket or a body member, such as may be formed for example by an aperture, hole, recess, cavity, channel, duct, or the like, which provides a liquid flow path when that gasket or body member is assembled with another or other body members to enclose the space.

In either of the above described two embodiments, there may be only three body members, namely the intermediate body member having the hole that provides the principal portion of the optical pathway and another body member on either side thereof within each of which is mounted an optically transparent window which is aligned with the hole when the body members are clamped together. If resilient gaskets are provided which contain the galleries, these are sandwiched between the three body members.

In other embodiments, the flow through cell may be formed by five body members, namely the intermediate body member having the hole, a transparent plate on either side thereof, possibly with resilient gaskets sandwiched therebetween, and a further body member on the "outer" sid of each

transparent plate. The further body members may contain holes aligned with the hole through the intermediate body member. In embodiments of the flow through cell having this combination of body members, the transparent plates provide the optically transparent windows.

Formation of the flow through cell from a plurality of body members, which may be formed for example from a corrosion-resistant metal such as for example titanium, or from an appropriate engineering plastic such as for example polyether ether ketone offers several advantages in that appropriately shaped body members can be readily manufactured by machining or moulding. The body members are not of complex shapes being generally of rectangular parallelopiped shape, and are readily clampable together, for example by machine screws or by through bolts with nuts, or by external clamps. The optically transparent windows can be provided by inserting windows of suitable materials into the body members as taught by H. T. Magnussen, Jr. in US Patent 5,062,706. Alternatively, as described above, plates of transparent material can be sandwiched between resilient gaskets and body members to provide windows. The use of resilient gaskets provides for sealing against leakage of flowing liquid, and is particularly advantageous for readily manufacturing a flow through cell to withstand high pressure liquid flows. Thus the invention provides a flow through cell that is relatively easily manufacturable.

The use of appropriately shaped galleries in the gaskets or alternatively in a body member abutting a hole in a gasket to direct flowing liquid through the optical pathway part of the flow through passage has been found to provide efficient sweeping or flushing of the optical pathway by successive increments of liquid with minimal mixing. The cross sectional area of a gallery carrying the liquid into, or out of, or between optical paths can be designed to increase or decrease the velocity of the liquid as appropriate for efficient sweeping or flushing of the optical pathway. Thus the invention allows for sensitive detection of light-absorbing substances in a flowing liquid stream while preserving th resolution of different substances present in consecutive parts of the flowing stream.

Preferably the body members also provide a second optical pathway through another part of the flow through passage. This other part of the flow through passage is preferably shorter in length than the first described optical pathway. This feature makes it possible to extend the range of concentration measurements by using two optical paths of different length.

The body members may also provide an optical pathway separated from the flow through passage for a reference beam to be passed through the cell. This optical pathway for passage of a reference beam can be constructed similarly to the optical pathway through the flow through passage such that its optical characteristics are similar.

Further features and advantages of the invention will become apparent from the following description with reference to the accompanying drawings of preferred embodiments thereof, which are given by way of non-limiting example only, for a better understanding of the invention and to show how it may be carried into effect.

Brief Description of the Drawings

Fig. 1 is a cross-section of an embodiment of the invention that provides a single optical path through the liquid stream and a separate optical path that allows passage of a reference beam.

Fig. 2 is a cross-section of an embodiment of the invention that provides two optical paths of different lengths through the liquid stream.

Fig. 3 illustrates examples of gaskets containing galleries for use in embodiments of the invention.

Fig. 4 illustrat s an annular spacer used in the embodiment of the invention shown in Fig. 2.

Fig. 5 shows a schematic cross section of part of an embodiment of the invention having flat transparent plates as windows.

Fig. 6 shows a schematic cross section of part of an embodiment of the invention having flat transparent plates as windows and having galleries formed in a body member rather than in a gasket.

Fig. 7 shows a schematic cross section of part of another embodiment of the invention having flat transparent plates as windows.

Detailed Description of Embodiments

Referring first to Fig. 1, the Illustrated flow through cell 100 is constructed from three body members 1, 2, and 3. Body member 2 has two parallel flat faces and includes four holes 8, 19, 25 and 26 extending through member 2 perpendicularly from one face thereof. Body members 1 and 3 are provided with parallel flat faces 27 and 28 respectively. To form cell 100 a gasket 4 made for example from a tetrafluoroethylene hexafluoropropylene copolymer ('FEP', E. I. du Pont de Nemours and Company) is placed as shown against flat face 27 of body member 1, body member 2 is placed as shown against gasket 4, a gasket 5 is placed as shown against the opposite flat face of body member 2, and flat face 28 of body member 3 is placed as shown against gasket 5. Machine screws 13 and 14 passing through holes 26 and 25 respectively and through corresponding holes in gaskets 4 and 5 secure the assembly by clamping the body members together as shown. Washer sets 16 and 15 (Belleville washers) in conjunction with screws 13 and 14 maintain sealing pressure of body members 1, 2 and 3 on gaskets 4 and 5.

Body member 1 is provided with a first optical window assembly 17 including a high pressure circumferential seal 17a (for example as taught in US Patent 5,062,706) and body member 3 is provided with a corresponding second optical window assembly 18. Said window assemblies provide transparent paths through the respective body members 1 and 3. Window assemblies 17 and 18 provide flat transparent surfaces that are level with surfaces 27 and 28

respectively. When cell 100 is assembled as shown window 17 aligns with a gallery 6 in gasket 4 and window 18 aligns with a gallery 7 in gasket 5. Galleries 6 and 7 align with the respectively adjacent ends of hole 8, so that an optical path A, A' is provided through cell 100 as shown. Body member 1 is provided with a liquid port 11 that is open to duct 12, which terminates as a hole in flat face 27. Gallery 6 in gasket 4 connects duct 12 to the adjacent end of hole 8. Similarly, body member 3 is provided with liquid port 9 that is open to duct 10, which terminates in a hole in flat face 28 and gallery 7 in gasket 5 connects duct 10 to the adjacent end of hole 8. A passage is thereby provided for the flow of liquid between liquid ports 9 and 11 through galleries 6 and 7 and hole 8. Liquid flowing through said passage is in optical pathway A,A' and spectrophotometric measurements can be made on the portion of liquid contained in hole 8 between window elements 17 and 18. The path length of light through said portion of liquid is defined by the distance between the corresponding flat faces 27 and 28 of body members 1 and 3 respectively. This distance is principally set by the length of hole 8, that is, by the thickness of body member 2. The volume of liquid in light path A,A' is principally defined by the length and diameter of hole 8.

As always in the design of flow cells for optical spectrometry, there is a trade off between providing a very small volume (which favours resolution) and providing sufficient light throughput to obtain an acceptable signal-to-noise ratio. The inventor has found that making hole 8 with a diameter of 1.4 mm and a length of 9.0 mm gives highly satisfactory performance with a specific spectrophotometer used by the inventor. These dimensions make the volume of hole 8 equal to 13.9 microlitres. The additional volume of the portions of galleries 6 and 7 at the respective ends of hole 8 brings the total volume of liquid in the optical path AA' to approximately 15 microlitres. Another useful set of dimensions for hole 8 is for example 0.5 mm diameter, 4.0 mm length. Those skilled in the art will appreciate that the dimensions of hole 8 must be selected with due consideration for the beam geometry of the spectrophotometer with which the cell is to be used. Dimensions stated herein are for example only.

A second optical path B,B' is provided through cell 100 parallel to A,A' by way of hole 19 in body member 2 and corresponding holes in body members 1 and 3 and gaskets 4 and 5. An optical spacer 20 is provided in hole 19, and window assemblies are provided in body members 1 and 3, so that the optical characteristics of second optical path B,B' are similar to those of optical path A,A. Second optical path B, B' is useful for the passage of a reference beam through cell 100. Such a reference beam is useful for spectrophotometric measurements of liquid in optical path A,A' as is known in the art. Lenses 21, 22, 23 and 24 form part of the corresponding window assemblies to focus light through the respective optical paths. In use cell 100 is placed in a spectrophotometer (not shown) in such a way that the sample beam of the spectrophotometer passes along A,A and the reference beam passes along B,B. To perform spectrophotometric measurements of a flowing liquid stream one of the two liquid ports 9 and 11 is connected to a source of flowing liquid such as the outlet of the column of a high performance liquid chromatograph. The other of the two liquid ports 9 and 11 is connected to an outlet duct so that the liquid stream can flow away in a controlled manner .

Referring now to Fig. 2, the illustrated flow through cell 200 is constructed from three body members 31, 32, and 33. Body member 32 has two parallel flat faces and includes five holes 38, 43, 44, 55, and 56 extending through body member 32 perpendicularly from one face thereof. Hole 44 is stepped and chamfered as shown to allow for the mounting of optical spacer/window assembly 63. Window assembly 63 includes a high pressure circumferential seal (for example, as taught in US Patent 5,062,706). Body members 31 and 33 are provided with parallel flat faces 57 and 58 respectively. To form cell 200 a gasket 34 is placed as shown against flat face 57 of body member 31, body member 32 is placed as shown against gasket 34, a gasket 35 is placed as shown against the opposite flat face of body member 32, and flat face 58 of body member 33 is placed as shown against gasket 35. Machine screws 13 and 14 passing through holes 56 and 55 respectively and through corresponding holes in gaskets 34 and 35 clamp the assembly together as shown. Washer sets 16 and 15 (Belleville washers) in conjunction with screws

13 and 14 maintain sealing pressure of body members 31, 32 and 33 on gaskets 34 and 35.

Body member 31 is provided with a first optical window assembly 17 and body member 33 is provided with a corresponding second optical window assembly 18. Said window assemblies provide transparent paths through the respective body members 31 and 33 and provide barriers to the passage of liquid. Window assemblies 17 and 18 provide flat transparent surfaces that are level with surfaces 57 and 58 respectively. When cell 200 is assembled as shown, first window 17 aligns with a first gallery 36 in gasket 34 and window 18 aligns with a gallery 37 in gasket 35. Galleries 36 and 37 align with the respectively adjacent ends of hole 38, so that a first optical path C, C' is provided through cell 200 as shown.

Body member 33 is provided with a liquid port 40 that is open to duct 39, which terminates as a hole in flat face 58. Gallery 37 in gasket 35 connects duct 39 to the adjacent end of hole 38. The opposite end of hole 38 is continuous with one end of first gallery 36 in gasket 34. The opposite end of gallery 36 is continuous with the end of hole 44. Body member 31 is provided with a third window assembly 68 providing an optical path through gallery 36 into hole 44. An annular spacer 66 (see Figure 4) in hole 44 seals against gasket 34 apart from the gap created by gallery 36. The opposite end of spacer 66 fits against window 70 of optical spacer/window assembly 63 in body member 32. Optical spacer/window assembly 63 provides an optical path through hole 71 in gasket 35 to a fourth optical window assembly 72 in body member 33. This provides a second optical path D,D' through cell 200. A portion of spacer 66 adjacent to optical spacer/window assembly 63 is cut away to provide a path 85 from the inside of spacer 66 to a second gallery 60 in gasket 36. Second gallery 60 provides a path to hole 43, which is continuous with hole 67 in gasket 35. Hole 67 is continuous with duct 42 that leads to liquid port 41 in body member 33. A passage is thereby provided for the flow of liquid between liquid port 40 and liquid port 41.

Liquid flows through said passage in optical pathways C,C' and D, D and separate spectrophotometric measurements can be made on the portion of liquid contained in hole 38 between window assemblies 17 and 18 and on the portion of liquid contained in that portion of the flow path between window assembly 68 and optical spacer/window assembly 63. The path length of light through the said portion of liquid in hole 38 is defined by the distance between the corresponding flat faces 57 and 58 of body members 31 and 33 respectively. This distance is principally set by the length of hole 38, that is, by the thickness of body member 32. The volume of liquid in light path C,C' is principally defined by the length and diameter of hole 38. The inventor has found that useful dimensions for hole 38 are for example length 9.0 mm, diameter 1.9 mm, and length 4.0 mm, diameter 2.0 mm when the cell was used with a specific spectrophotometer. Those skilled in the art will appreciate that the dimensions of hole 38 must be selected with due consideration for the beam geometry of the spectrophotometer with which the cell is to be used. Dimensions stated herein are for example only. The path length of light through the said portion of liquid between window assembly 68 and optical window/spacer assembly 63 is principally defined by the distance between windows 68 and window 70 of optical spacer/window assembly 63. This distance is principally set by the thickness of annular spacer 66. The length of window 70 must be such as to provide a firm seal between annular spacer 66 and gasket 34 when annular spacer 66 is pressed against gasket 34 by window 70. The volume of liquid in light path D,D' is principally defined by the length and diameter of the space 65 in annular spacer 66 and that portion of gallery 36 between windows 68 and 70. The inventor has found that a suitable annular spacer 66 has an internal diameter of 2.4 mm and a thickness of 1 mm. This is useful when hole 38 has an internal diameter of 1.9 mm and a length of 9 mm, because the optical path lengths through the flowing liquid C,C' and D,D' are then in the ratios 9:1. Those skilled in the art will appreciate that the dimensions of space 65 must be selected with due consideration for the beam geometry of the spectrophotometer with which the cell is to be used. Dimensions stated herein are for example only.

In use cell 200 is placed in a special spectrophotometer (not shown) that is provided with two sample beams, as is known in the art (see for example US Patent 5,214,593). Cell 200 is located in such a way that one sample beam of the spectrophotometer passes along C,C' and the other sample beam passes along D,D'. The optical path length through the liquid flow path through C,C' is much greater than that through D,D'. The absorbance is proportional to the optical path length as well as to the concentration of the absorbing species. Consequently it is possible to extend the range of concentration measurements by using two optical paths of different length, as is known. To perform spectrophotometric measurements of a flowing liquid stream, liquid port 40 is connected to a source of flowing liquid such as the outlet of the column of a high performance liquid chromatograph, while liquid port 41 is connected to an outlet duct so that the liquid stream can flow away in a controlled manner.

Figure 3 shows various shapes for galleries 6 and 7 (in gaskets 4 and 5) that have been tested by the inventor. In all of them the wider ends of galleries 6 and 7 open into hole 8 when body 100 is assembled as previously described with reference to Figure 1. The narrower end of each gallery 6 and 7 opens into the associated duct 12 or 10 respectively. See Figure 1 for details of ducts 10 and 12. The shapes shown for galleries 6 and 7 were tested to determine whether or not the different flow characteristics that were expected to result from the different shaped galleries had any effect on the performance of the flow cell. No such effect was found. It was concluded that all of the different shapes shown provided adequate flushing of the cell and that the action of the liquid entering or leaving the cell immediately adjacent to the windows was more important than the subtle details of the flow. This conclusion, while valid for the flow rates tested, might need to be modified for other flow rates.

Figures 5, 6 and 7 are to be understood as showing schematic cross sections of parts of alternative embodiments of the invention including one optical path. It is to be understood that means (not shown) are provided to keep the parts properly held together to form a cell as indicated in the foregoing description, and it is further to be understood that any such cell can contain more than one optical path as also indicated in the foregoing description.

Referring to Figure 5, cell 300 is made up of body members 301, 302 and 303, transparent plates 311 and 312 and resilient gaskets 304 and 305. An optical path A, A' passes through hole 308 in body member 302. Hole 308 forms part of a small volume through passage that extends from liquid port 309 in body member 303 through gallery 307 in gasket 305, through hole 308 and gallery 306 in gasket 304 to liquid port 310 in body member 301. A disadvantage of this method of constructing the invention is the need to drill holes 313 and 314 through transparent plates 312 and 311 respectively. This can be avoided by use of alternative embodiments as indicated in Figures 6 and 7.

Referring to Figure 6, cell 500 is made up of body members 501, 502 and 503, transparent plates 511 and 512 and resilient gaskets 504 and 505. An optical path A, A' passes through hole 508 in body member 502. Hole 508 forms part of a small volume through passage that extends from liquid port 509 in body member 502 through gallery 507 in body member 502, through hole 508 and gallery 506 in body member 502 to liquid port 510 in body member 502. A possible disadvantage of this method of constructing the invention is that the entry points of galleries 506 and 507 into hole 508 are not immediately adjacent to transparent plates 511 and 512 but are substantially adjacent to the plates 511 and 512 being separated therefrom by the thickness of resilient gaskets 504 and 505 respectively. This is expected possibly to reduce to some extent the efficiency of flushing the optical cell formed between transparent plates 511 and 512 by liquid flowing between liquid ports 509 and 510. This can be avoided by use of an alternative embodiment as indicated in Figure 7. Yet another way of avoiding this possible disadvantage is to make body member 502 out of a suitably resilient material so that gaskets 504 and 505 can be eliminated

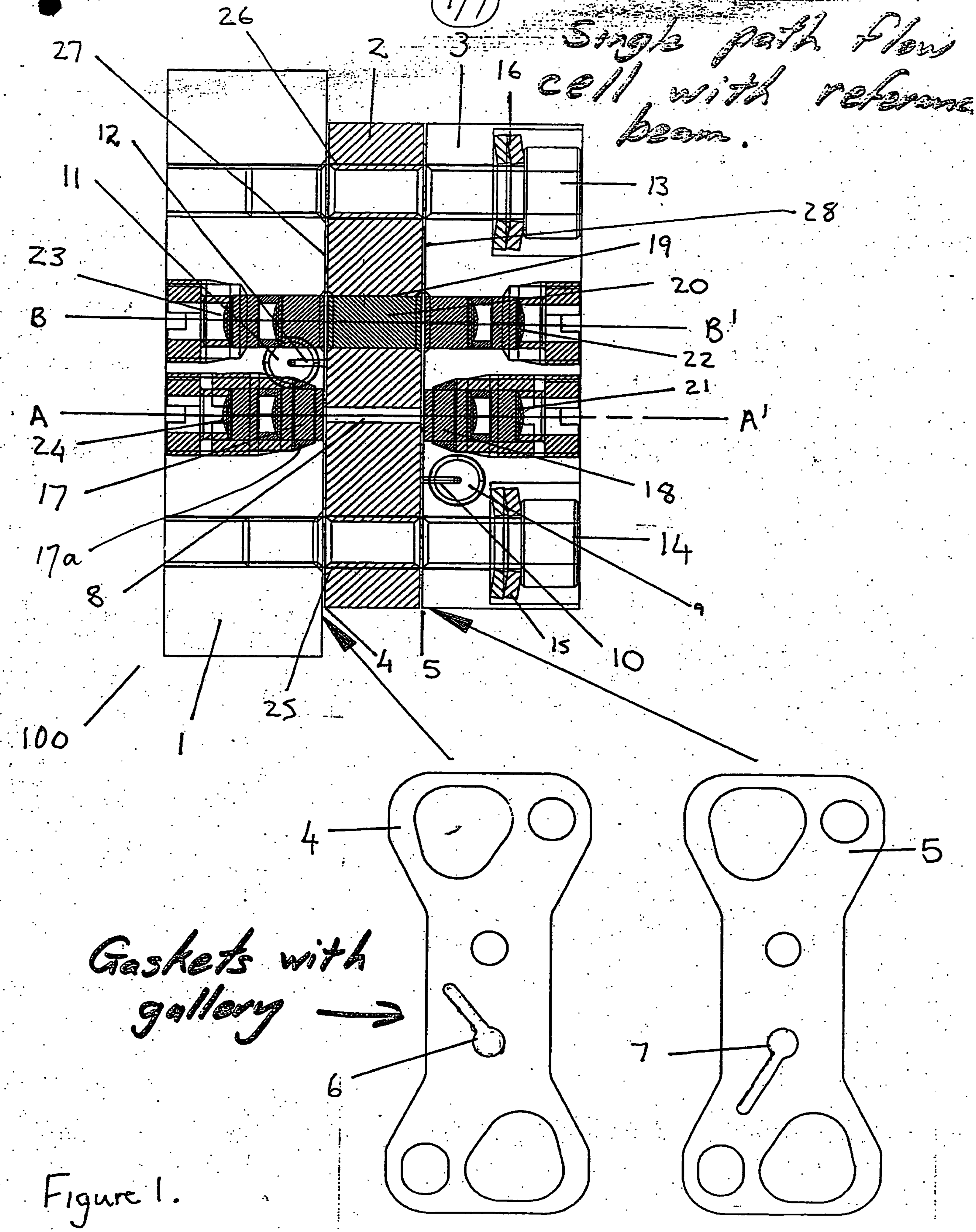
Referring to Figure 7, cell 600 is made up of body members 601, 602 and 603, transparent plates 611 and 612 and resilient gaskets 604 and 605. An optical path A, A' passes through hole 608 in body member 602. Hole 608 forms part of a small volume through passage that xtends from liquid port 609

in body member 602 through gallery 607 in gasket 605, through hole 608 and gallery 606 in gasket 604 to liquid port 610 in body member 602.

The improvement in resolution obtained by the use of a cell according to the invention is indicated by the results of an experiment in which the effluent of a high performance liquid chromatograph was analysed using a prior art cell (a Varian Prostar 310 15 microlitre flow cell) and then the same sample was analysed under the same conditions using a 15 microlitre flow cell according to the invention . The width at half height of the peak obtained with the prior art cell was 9.7 microlitres while that with the cell according to the invention was only 7.0 microlitres. The smaller the width at half height, the better the resolution. The figure-of-merit that defines the resolution is called the variance. This was 16.9 microlitres squared for the prior art cell and 8.84 microlitres squared for the cell according to the invention.

The invention described herein is susceptible to variations, modifications and/or additions other than those specifically described and it is to be understood that the invention includes all such variations, modifications and/or additions which fall within the spirit and scope of the above description.

DATED: 12 November, 2002
PHILLIPS ORMONDE & FITZPATRICK
Attorneys for:
VARIAN AUSTRALIA PTY LTD


Single path flow cell with reference beam.
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Gaskets with gallery
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Figure 1.

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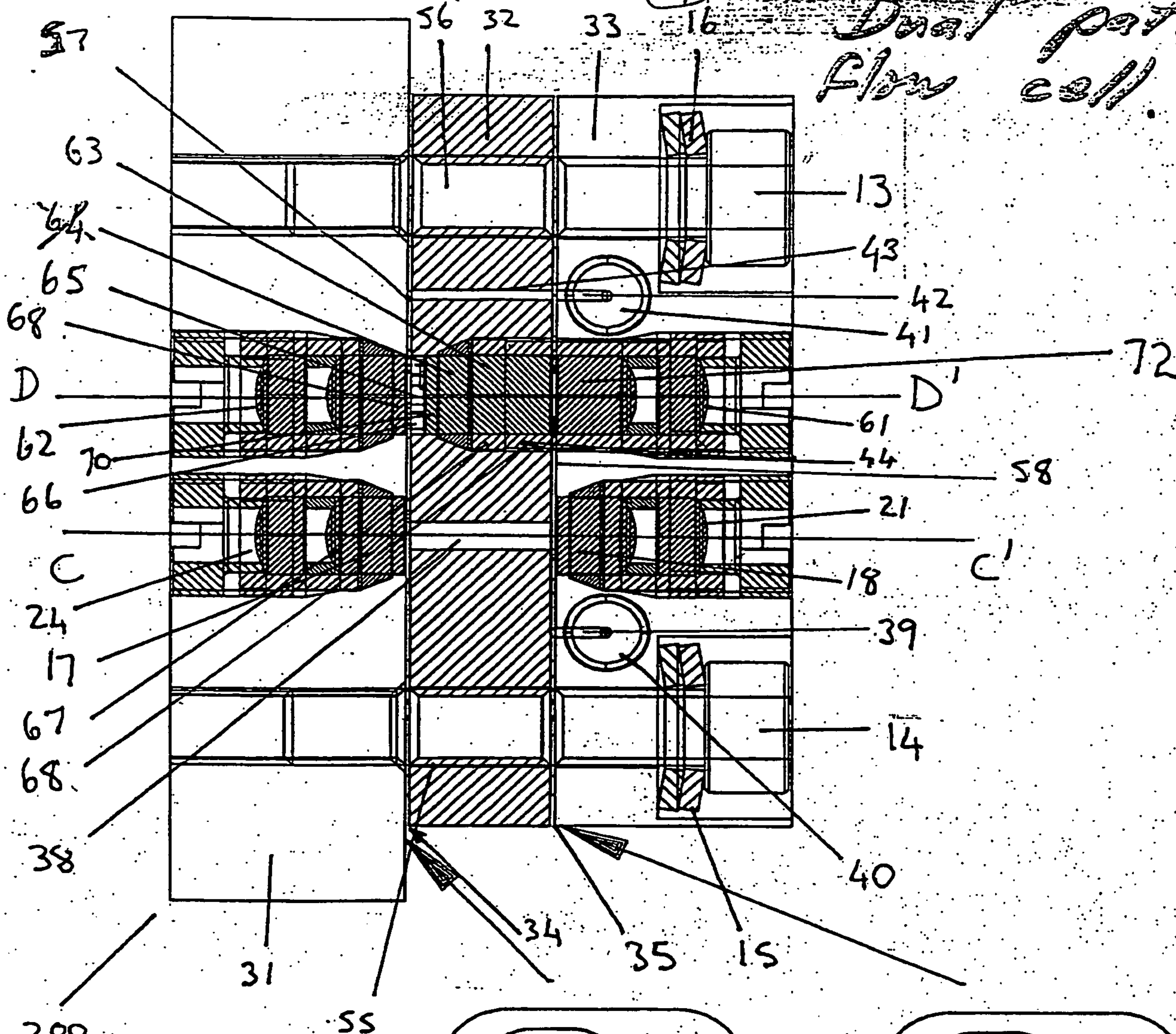
Dual path flow cell.



Gaskets with gallery

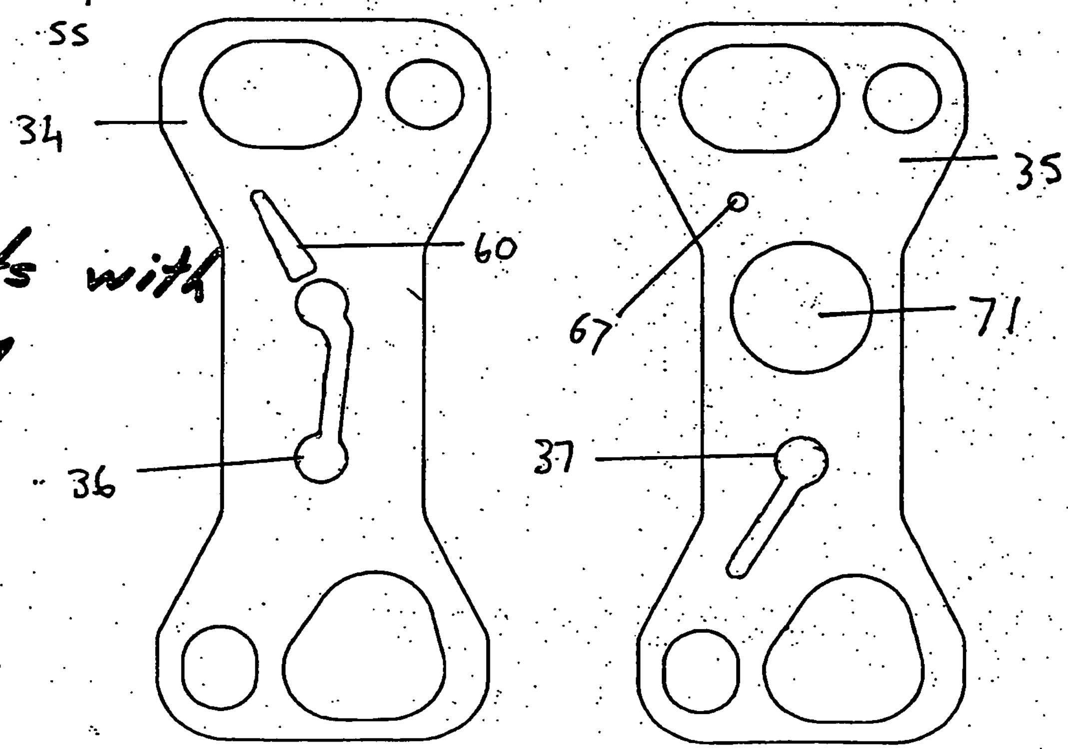


Figure 2.

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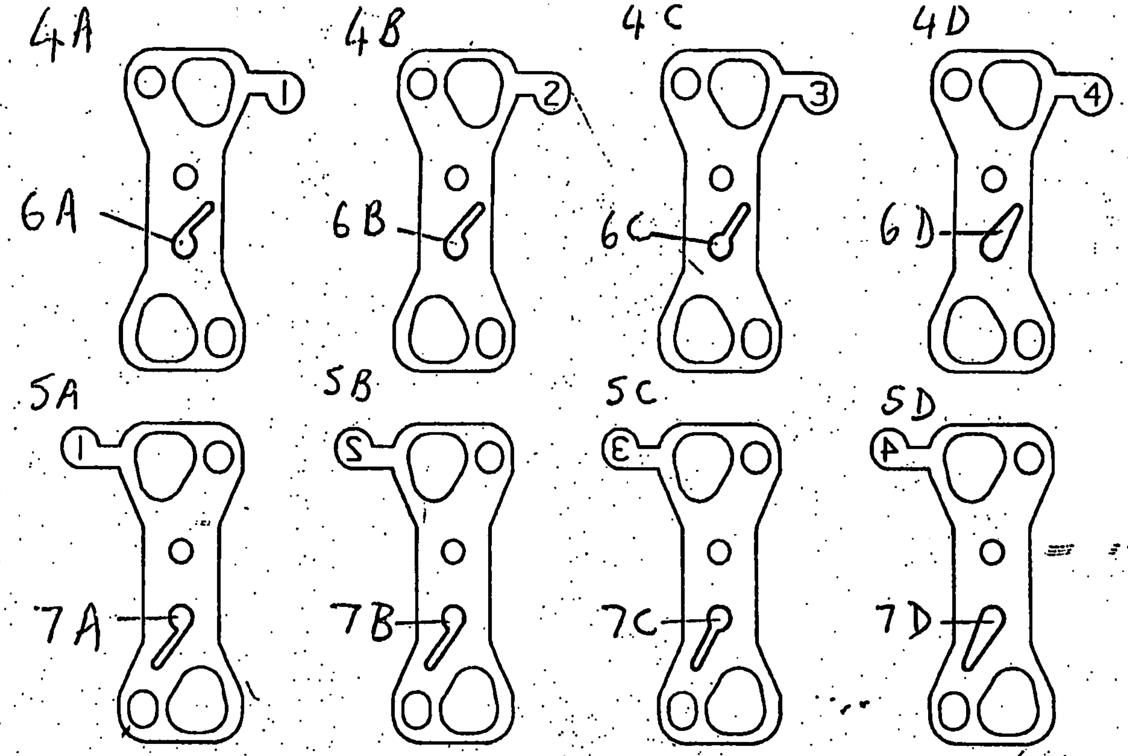

Various gallery designs for a single path flow cell.

Figure 3.

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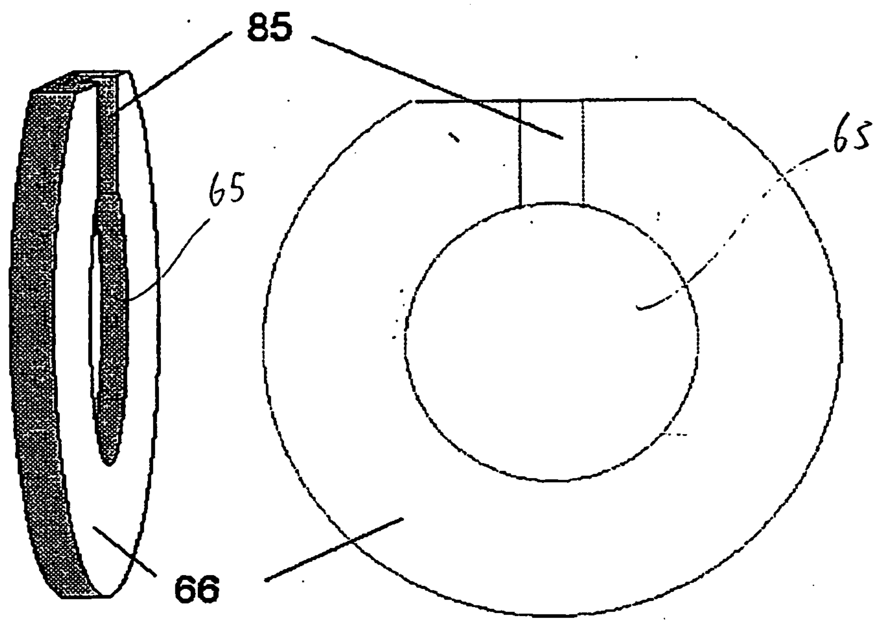

Figure 4.

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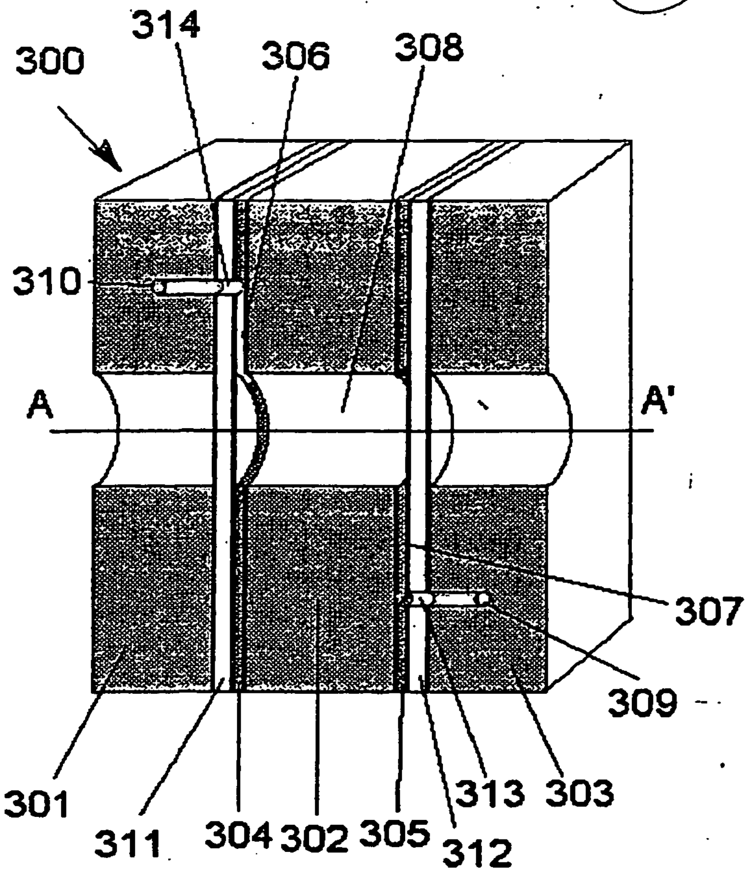

Figure 5.

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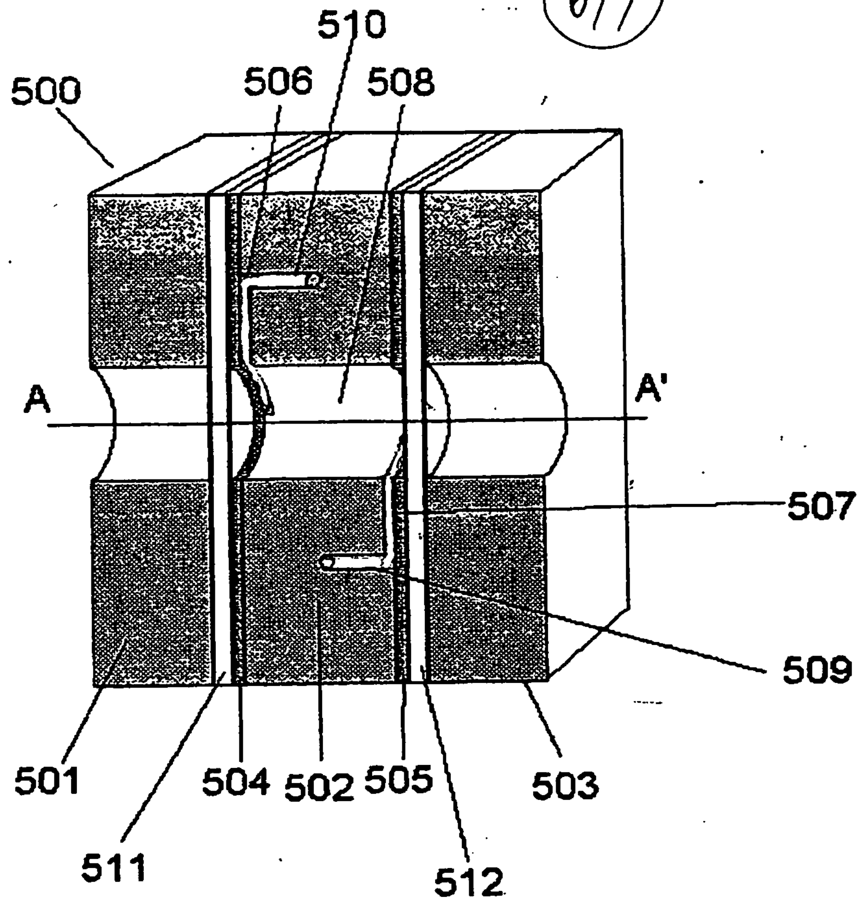

Figure 6.

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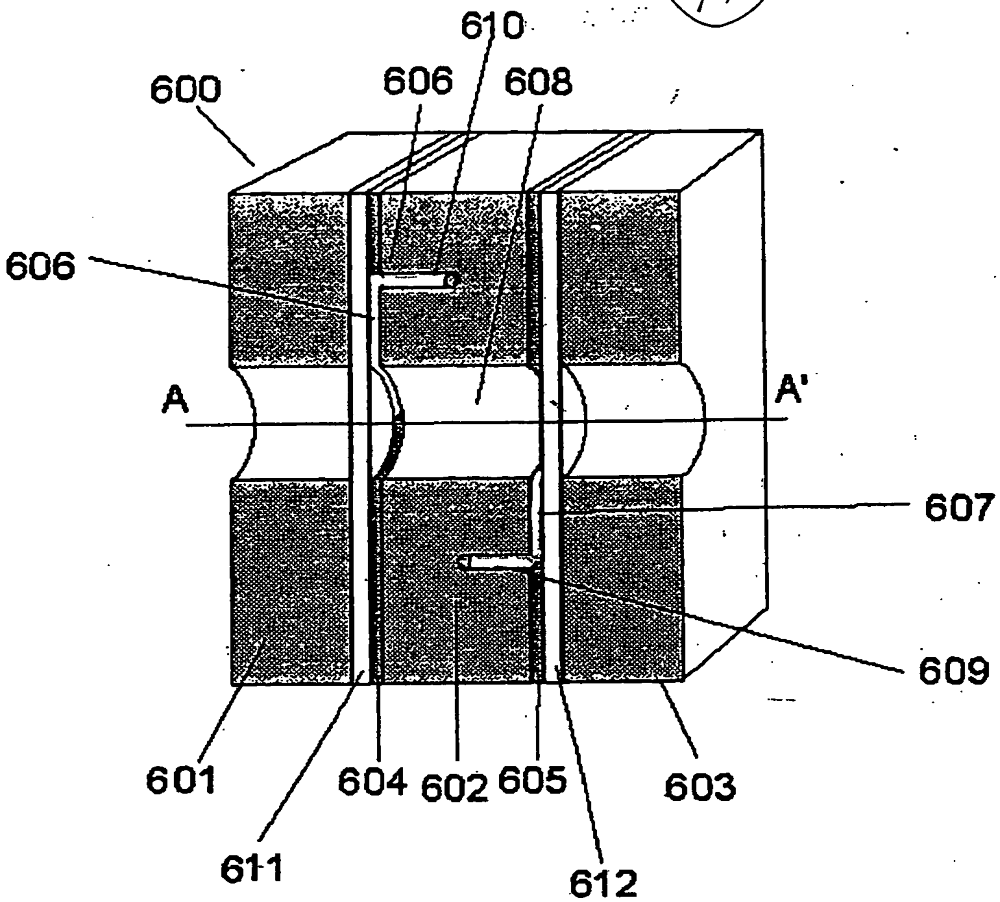

Figure 7.